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Carbon black having an immunologically-active compound bound thereto

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CARBON BLACK HAVING AN IMMUNOLOGICALLY-ACTIVE COMPOUND BOUND THERETO

Background of the Invention

Various methods for detecting the presence of an analyte in a sample of biological fluid through the use of immunochemistry have been described. In the so-called "sandwich" method, for example, a target analyte such as an antigen is "sandwiched" between a labeled antibody and an antibody immobilized onto a solid support. The assay is read by observing the presence and amount of bound antigen-labeled antibody complex. In the competition immunoassay method, antibody bound to a solid surface is contacted with a sample containing an unknown quantity of antigen analyte and with labeled antigen of the same type. The amount of labeled antigen bound on the solid surface is then determined to provide an indirect measure of the amount of antigen analyte in the sample.

Because these and other methods discussed below can detect both antibodies and antigens, they are generally referred to as immunochemical ligand-receptor assays or simply immunoassays.

Solid phase immunoassay devices, whether sandwich or competition type, provide sensitive detection of an analyte in a biological fluid sample such as blood or urine. Solid phase immunoassay devices incorporate a solid support to which one member of a ligand-receptor pair, usually an antibody, antigen, or hapten, is bound. Common early forms of solid supports were plates, tubes, or beads of polystyrene which were well known from the fields of radioimmunoassay and enzyme immunoassay. More recently, a number of porous materials such as nylon, nitrocellulose, cellulose acetate, glass fibers, and other porous polymers have been employed as solid supports.

A number of self-contained immunoassay kits using porous materials as solid phase carriers of immunochemical components such as antigens, haptens, or antibodies have been described. These kits are usually dipstick, flow-through, or migratory in design.

In the more common forms of dipstick assays, as typified by home pregnancy and ovulation detection kits, immunochemical components such as antibodies are bound to a solid phase. The assay device is "dipped" for incubation into a sample suspected of containing unknown antigen analyte. Enzyme-labeled antibody is then added, either simultaneously or after an incubation period. The device next is washed and then inserted into a second solution containing a substrate for the enzyme. The enzyme-label, if present, interacts with the substrate, causing the formation of colored products which either deposit as a precipitate onto the solid phase or produce a visible color change in the substrate solution. Baxter et al., EP-A 0 125 118, disclose such a sandwich type dipstick immunoassay. Kali et al., EP-A 0 282 192, disclose a dipstick device for use in competition type assays.

Flow-through type immunoassay devices were designed to obviate the need for extensive incubation and cumbersome washing steps associated with dipstick assays. Valkirs et al., U.S. Patent No. 4,632,901, disclose a device comprising antibody (specific to a target antigen analyte) bound to a porous membrane or filter to which is added a liquid sample. As the liquid flows through the membrane, target analyte binds to the antibody. The addition of sample is followed by addition of labeled antibody. The visual detection of labeled antibody provides an indication of the presence of target antigen analyte in the sample.

Korom et al., EP-A 0 299 359, discloses a variation in the flow-through device in which the labeled antibody is incorporated into a membrane which acts as a reagent delivery system.

The requirement of multiple addition and washing steps with dipstick and flow-through type immunoassay devices increases the likelihood that minimally trained personnel and home users will obtain erroneous assay results.

In migration type assays, a membrane is impregnated with the reagents needed to perform the assay. An analyte detection zone is provided in which labeled analyte is bound and assay indicia is read. See, for example, Tom et al., U.S. Patent No. 4,366,241, and Zuk, EP-A 0 143 574.

The sensitivity of migration type assays is frequently reduced, however, by the presence or formation in the sample of undesirable solid components which block the passage of labeled analyte to the detection zone. Assay sensitivity also declines when migration assay devices are flooded with too much liquid sample.

Migration assay devices usually incorporate within them reagents which have been attached to colored labels, thereby permitting visible detection of the assay results without addition of further substances. See, for example, Bernstein, U.S. Patent No. 4,770,853, May et al., WO 88/08534, and Ching et al., EP-A 0 299 428.

Among such labels are gold sol particles such as those described by Leuvering in U.S. Patent No. 4,313,734, dye sol particles such as described by Gribnau et al., in U.S. Patent No. 4,373,932 and May et al., WO 88/08534, dyed latex such as described by May, supra, Snyder, EP-A 0 280 559 and 0 281 327, and dyes encapsulated in liposomes by Campbell et

al., U.S. Patent No. 4,703,017. These colored labels are generally limited in terms of the immobilization methods which are suitable. Moreover, they require a relatively large amount of ligand molecule and can involve expensive reagents, thereby adding to the cost.

In our Application No. 9026221 (GB2239313) we describe and claim an immunochemical assay device comprising: a base member; an array disposed on said base member, said array comprising:

(i) a reservoir pad having sufficient porosity and volume to receive and contain a liquid sample on which the assay is to be performed;

(ii) a wicking membrane disposed distally to said reservoir pad, said wicking membrane having sufficient porosity and volume to absorb a substantial proportion of the sample received in said reservoir pad; and

(iii) at least one filter zone interposed between and contiguous with said wicking membrane and said reservoir pad, said filter zone being (a) contiguous across a surface of said reservoir pad which is sufficiently small with respect to the volume of said reservoir pad to meter the passage of the liquid sample from said reservoir pad to said filter zone and (b) operable to permit passage of any specific ligand-receptor complex in said sample from said reservoir pad to said wicking membrane while impeding passage of larger components then contained in said sample; and (iv) at least one immobilized substance disposed in at least one zone of said wicking membrane and defining assay indicia, said immobilized substance being operable to bind a specific ligand-receptor complex contained in the sample to form said assay indicia.

The present invention relates to one immunochemical label which is particularly well-suited for use in the foregoing device but which can be used in other immunological assays as well, in particular an immunochemical label in which to an immunological ligand or ligand binding molecule is linked directly or indirectly to the surface of finely particulate carbon black. According to the present invention there is provided an immunochemical label comprising particulate carbon black on which is adsorptively immobilized a component which terminates distally from the point of adsorption with an immunologically active ligand or ligand binding molecule, for reaction between immunological ligand or ligand binding molecules and an analyte.

The immunological label can be diagrammatically depicted as C~X:L in which C is the finely particulate carbon black, "~" represents an adsorption linkage, L is a component containing a ligand- or ligand binding unit, X is a linking agent, and ":" represents a covalent bond.

The ligand- or ligand binding unit L can be bound to a bridging member either covalently or immunologically (herein designated by "*"). For example, the ligand- or ligand binding unit can be covalently bound to a linking agent such as glutaraldehyde which in turn is covalently bound to a proteinaceous bridging member such as bovine serum albumin (BSA) which in turn is adsorbed on the carbon. Likewise, avidin or streptoavidin can be linked through biotin to the ligand- or ligand binding molecule and the avidin or streptoavidin adsorbed on the carbon particles. Alternatively a primary antibody, serving as the ligand- or ligand binding unit is immunologically bound to a secondary antibody and the secondary antibody is adsorbed to the carbon particles. Typical structures of the C~X:L embodiment thus include:

C~{protein:X:ligand},
C~{protein:X:ligand binding molecule},
C~{2°Ab*1°Ab}, and
C~{protein:X:2°Ab*1°Ab}.

In one embodiment, a linking agent Y is both adsorbed on the carbon particle and covalently bound to the ligand- or ligand binding unit to form a label of the general formula

C~Y:L. The linking agent Y can be a single molecular species, Y', as more fully discussed below, or can be a composite such as linking agent:protein:linking agent:

C~Y':{ligand},

C~Y':{ligand binding molecule},

C~Y':protein:X:{ligand}, and

C~Y':protein:X:{ligand binding molecule}.

Thus a member of a particular class of organic compounds serving as a linking agent is adsorbed on the carbon particles and covalently bound to a ligand, ligand binding molecule, or protein.

The foregoing carbon sols can be prepared by a number of methods. The full, non-carbon particle structure such as {protein:X:ligand}, {protein:X:ligand binding molecule}, {2°Ab*1°Ab}, or {protein:X:2°Ab*1°Ab} can be prepared and then added to a suspension of the carbon particles for adsorption. Alternatively, a terminal portion of the non-carbon particle structure first can be adsorbed on the carbon particles and the remainder of the non-carbon particle structure then introduced chemically. For example, a protein such as bovine serum albumin, avidin, or streptoavidin can be adsorbed on the carbon particles and then linked, using for example glutaraldehyde for bovine serum albumin or biotin for avidin or streptoavidin, to the ligand or ligand binding molecule. Similarly, a 2°antibody can be adsorbed on the carbon particles and a 1°antibody then joined immunologically.

Linking reagent Y' suitable for covalently-linking ligand and ligand binding molecules such as haptens, antigens, or antibodies, or for covalently-linking protein bridging groups, include imides, azides, isothiocyanates, imidoesters, and dialdehydes, as for example, maleimide, succinimide, phenylazide, glutaraldehyde, N-hydroxysuccinimide ester, phenylisothiocyanate, 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid, 4-N,N-dimethylaminoazobenzene-4'-isothiocyanate, flourescein isothiocyanate and rhodamineisothiocyanate.

The complete non-carbon particle structure, prepared by reacting the ligand and (or ligand binding molecule), any bridging protein, and linking agent, can be adsorbed on the surface of the finely particulate carbon black. Alternatively, the linking reagent alone first can be adsorbed on the finely particulate carbon black and then covalently bound to the ligand, ligand binding molecule, and/or bridging protein.

In any of the above procedures, it generally is desirable to add a suspending adjuvant to the aqueous suspension of the finely particulate carbon black, for example a polyalkylene glycol or polysaccharide. As will be seen below, similar substances subsequently are added as a protective agent after linking the immunological ligand or ligand binding molecules to the finely particulate carbon black. The amount added at this stage thus is relatively small, generally being that sufficient merely to assist in the suspension of the carbon particles.

The linking reagent then is allowed to both (i) react covalently with the immunological ligand or ligand binding molecules and (ii) be adsorbed on finely particulate carbon black, either simultaneously or sequentially. While dependent on the particular linking reagent, the linking reaction

generally is conducted over several hours at pH values of from about 7.0 to about 9.5.

A variety of commercially available finely particulate carbon black materials can be used such as Monarch(RTM) 1,000, 120, or 880, Vulcan(RTM) XC72 or XC72R, or Regal(RTM) 250R or 500R. The suitability of any particular source can be readily determined by homogenating the material in buffer and measuring the optical density.

Preferably, the finely particulate carbon black with the ligand or ligand binding molecule bound covalently or passively is treated with a polyalkylene glycol or polysaccharide protective agent to minimize hydrophobicity and maximize dispersability. Suitable materials for such coating are polyethylene glycols having a molecular weight of from 100 to 20,000, preferably from 5,000 to 12,000, and protective polysaccharides such as dextran having a molecular weight of from 10,000 to 500,000, preferably from 10,000 to 50,000. This coating can be readily achieved by contacting the linked carbon black with a 0.5% to 5% weight/volume aqueous solution of the polyethylene glycol or dextran.

In a further embodiment, the immunochemical label is treated with at least one biologically acceptable ionic or nonionic surfactant, such as long chain alkyl trimethylammonium salt, sodium deoxycholate, Tritons(RTM) and Tweens(RTM), typically in a concentration range of from 0.01 to 0.5%. After each such treatment, of which there can be several, with the same or different types of detergent, the immunochemical label is washed to remove excess detergent.

The resulting immunochemical label then can be suspended in an aqueous media. Such aqueous suspensions of the immunochemical label are particularly useful for the fabri-

cation of immunoassay devices, both those of the present invention and those of other structures. Preferably the aqueous suspension includes at least one buffer in order to provide a pK_a at which the labelled immunological ligand or ligand binding molecule is stable; e.g. within the range of from 6 to 9 and preferably from 6.5 to 8.5

The following Examples further illustrate the present invention.

Sensitivity Procedure

Sensitivities are determined in the following examples by preparing standard solutions of human chorionic gonadotropin in concentrations of 25 mIU/ml, 50 mIU/ml, 75 mIU/ml, and 100 mIU/ml. Samples (0.15-0.20 ml) of the standard are applied to the assay device and sensitivity determined by the ability of the device to detect a given concentration of human chorionic gonadotropin.

Example 1

A. Ten milligrams of Vulcan XC72 carbon particles are homogenized in 2 ml of 20 mM Tris-hydrochloride buffer (pH 6.8) containing 40 mM sodium chloride and 2% dextran 9,400. After 2 hours incubation at ambient temperature, a solution of 5 mg of fluorescein isothiocyanate in 1 ml of Tris-hydrochloride buffer is added to the solution. The mixture is briefly sonicated and incubated for approximately 12 hours at ambient temperature. After incubation, 20 ml of 0.1 M sodium phosphate buffer (pH 7.6) in 0.1 M sodium chloride are added to the carbon solution which then is centrifuged at 4°C at 15,000 RPM. This step is repeated three times and the resultant pellet suspended in 20 ml of phosphate buffer. After brief sonication, 3 mg of a monoclonal antibody made against human chorionic gonadotropin are added to the suspension, and the mixture incubated for 6 hours at ambient temperature. The mixture then is centrifuged three times at 15,000 RPM to remove unreacted antibody. The final pellet is suspended in 20 ml of 0.1 M Hepes buffer (pH 7.5) containing 1% bovine serum albumin, 5% sucrose, 0.1M sodium chloride, and 0.05% sodium

azide. Cetyltrimethyl ammonium bromide is added until a final concentration of 0.025% is achieved. This then is incubated for 30 minutes and centrifuged at 15,000 RPM. The resultant pellet is suspended in 20 ml of 0.1 M Hepes buffer (pH 7.5) containing 1% bovine serum albumin, 5% sucrose, 0.1M sodium chloride, and 0.05% sodium azide, sonicated briefly, and diluted with sodium deoxylate to a final concentration of 0.1%, after which it is incubated for 30 minutes at ambient temperature and recentrifuged. The pellet again is suspended in 20 ml of 0.1 M Hepes buffer (pH 7.5) containing 1% bovine serum albumin, 5% sucrose, 0.1M sodium chloride, and 0.05% sodium azide and sonicated briefly.

B. Preparation of Device. A sample of preactivated nylon membrane (Pall Immunodyne (RTM)) with a pore size of 5 μm is cut to 180mm x 25mm size and attached to the bottom of a thin plastic plate (100mm x 180mm) as the wicking membrane. An assay indicia zone of immobilized antibody is defined on the membrane by spraying 36μl of a solution of 3 mg/ml sheep anti-human chorionic gonadotrophin (hCG) antibody in 0.1 M sodium phosphate buffer (pH 7.6) and 5% sucrose in a line approximately 1.5 cm from the bottom using a Camag Linomat IV. After spraying, the membrane is dried at 37°C for 30 minutes and then treated with a solution of 2% nonfat dry milk (Carnation (RTM)) and 2% sucrose in 0.1M sodium phosphate buffer. The membrane then is washed with 2% sucrose in 0.1 sodium phosphate and allowed to stand at ambient temperature for approximately 12 hours for further drying. The base and wicking membrane can be stored in a desiccator until further processed.

Two cellulose membranes (Whatman (RTM) ET31) are pretreated with a solution of 0.1 M sodium phosphate buffer (pH 7.4), 0.1% bovine serum albumin, 0.5% nonfat dry milk, 2% sucrose,

and 0.05% sodium azide and then incubated for 30 minutes at ambient temperature.

The second filter element is prepared by drying the 2 pretreated cellulose membranes in a vacuum desiccator for 1 hour at ambient temperature.

The first filter element is prepared by incubating a rectangular piece of cellulose membrane (Schleicher & Schuell (RTM)) measuring 5 mm x 180 mm at ambient temperature for 30 minutes in a solution of the carbon sol. The membrane then is placed on a glass plate and heat dried at 36°C under a constant vacuum in a lyophilizer and stored dry in a desiccator until use.

The first filter element is attached adjacent to the second filter element and the second filter element is attached to the plastic base adjacent to the wicking membrane. The plastic plate then is cut into a plurality of strips 100 mm in length and 7.5 mm in width so that each contains a linear array of reservoir pad, first filter element, second filter element and wicking membrane.

The test strips are dried in a vacuum drier for about an hour and stored in a desiccator at ambient temperature until use.

To carry out human chorionic gonadotropin or luteinizing hormone assays, 100 µl of urine sample is dispensed in a culture tube and the strip is then inserted into the tube Upon contact with the urine sample, the carbon particle-antibody conjugates immediately become solubilized and migrate toward the wicking membrane. A positive test corresponds to an intense color of the carbon black particles concentrated in the indicia. The detection limit is about 25 mIU/ml in both the human chorionic gonadotropin and luteinizing hormone assays.

Example 2

A strip is prepared according to the procedure of Example 1B omitting the first filter element.

The test strip then is inserted into a test tube containing the carbon sol labeled antibody (5 µl), and a urine sample containing human chorionic gonadotropin (100 µl)which are mixed thoroughly. A detectable signal begins to appear after about 1 minute. The sensitivity of this assay measured about 25 mIU/ml.

Example 3

Carbon sol reagents coated with monoclonal antibodies against human chorionic gonadotropin or luteinizing hormone (5 µl per tube) are lyophilized. The tubes can be stored in a desiccator at ambient temperature until use.

To conduct human chorionic gonadotropin or luteinizing hormone assays, 100 µl of urine sample are dispersed in a culture tube containing the dried or lyophilized carbon sol. The carbon reagent immediately goes into solution upon the contact with a urine sample. A test strip prepared as in Example 1B but without the first filter element and reservoir pad and on which has been sprayed a line of sheep anti-whole human chorionic gonadotropin antibody (3 µg per

strip) as the indicia then is inserted into the tube. When the migrating sample mixture reaches the indicia, a black band begins to appear if the urine sample contained human chorionic gonadotropin or luteinizing hormone. The sensitivity of the assays using the dried or lyophilized carbon reagent is about 25 mIU/ml in both cases. The dried or lyophilized carbon reagent remains active, showing the same sensitivity following storage for over a year at ambient temperature.

Example 4

An assay device was prepared according to Example 1B eliminating the first filter element and using 1 mg/ml of anti-thyroxine antibody as the line spray on the wicking membrane.

Upon insertion in a mixture of 5 μ of carbon sol linked to thyroxine (see Example 15) and 100 μl of serum (competitive assay), the control band begins to appear in about two minutes. At thyroxine (unlabeled) levels in the serum sample higher than about 60 ng/ml, no band formation occurs (faint band appears at 59 ng/ml). In contrast, to produce a band as strong as the control band in the absence of thyroxine in the serum sample, less than 10 ng/ml of thyroxine is required.

Example 5

An assay device was prepared according to Example 1B eliminating the first filter element and using 2 mg/ml of commercially available Lymes antigen (OEM Concepts) as the line spray on the wicking membrane.

When the reservoir end of the device is dipped into a mixture of 100 μl of human serum and 5μl of carbon particles

Immunoglobin G (see Example 15) in 20 mM ethylenediaminetetraacetate.

A detectable signal appears in about 3 minutes if the sample is seropositive.

Example 9

An assay device was prepared according to Example 5 and an additional line of Rubella antigen was sprayed approximately 7 mm from and parallel to the line of lyme antigen.

A Rubella seropositive sample (10 µl) was spotted on the second filter element. The device was inserted into a tube containing a 100 µl suspension of carbon particles (10 µl) labeled with flourescein isothiocyanate conjugated goat anti-human Immunoglobin G (see Example 15) in 20 mM ethylenediamine tetraacetate.

A detectable signal appeared in about 5 minutes along the line of Rubella antigen.

Example 10

The procedure in Example 9 was followed except that a Rubella and Lyme seropositive sample (20 µl) was spotted on the membrane. Two detectable signals began to appear in about 5 minutes.

Example 11

The suitability of different carbon materials for preparation of the carbon sols and buffers for the same can be readily determined by the following techniques.

A. Mixtures of 5 mg of different carbon black (Monarch 1,000, Monarch 880, Monarch 120, Regal 250R, Regal 500R, Vulcan XC72R, and Vulcan XC72, all obtained from Cabot) and 100 µl of 2% polyethylene glycol (6,000-8,000) are ground for 5 min. and diluted up to 10 ml with phosphate saline buffer containing 2 mg of a monoclonal antibody made against human chorionic gonadotropin. After a brief sonication to disperse the carbon particles in the monoclonal antibody solution, the mixtures are incubated for 6 hours at ambient temperature with stirring. At the end of the incubation, the sample is washed three times by centrifugation to remove any excess antibody. Each centrifugation is carried out at 15,000 RPM for 20 min. using 10 ml of phosphate buffer solution. The final pellet is suspended in 10 ml of 3% phosphate buffer solution and sonicated briefly to ensure complete dispersion of the carbon particles.

For human chorionic gonadotropin assay, 20 µl of the carbon sol and 200 µl of urine sample are dispersed and mixed well in a culture tube (10 x 75 mm). The mixture is then allowed to migrate into a strip of Whatman paper (31ET) measuring 5 mm in width and 100 mm in height, line-sprayed with sheep anti-human chorionic gonadotropin antibody and blocked with 1% bovine serum albumin in phosphate buffer solution (pH 7.4).

Vulcan XC72 appears to give the best signal-to-noise ratio at 200 mIU/ml human chorionic gonadotropin. Similar results are obtained with Vulcan XC72R, but the positive signal is slightly lower.

B. Five milligrams of the same carbon blacks are suspended in 2 ml of 20 mM Tris-HCl buffer (pH 6.8) containing 40 mm sodium chloride and 2% (w/v) dextran 9,400 by homogenization. After 2 hours of incubation at ambient temperature, 1 ml of 3% bovine serum albumin solution is

added to the homogenized carbon suspension. The mixture is sonicated briefly and incubated further for approximately 12 hours at ambient temperature. At the end of the incubation, 5 μl of the mixture is dispensed in a cuvette containing 1 ml of distilled water. Absorbency at 700 nm is measured for each sample. The results are as follows:

Source of Carbon Black	OD at 700 nm
Monarch 1,000	0.2333
Monarch 880	0.3129
Vulcan XC72R	0.6878
Vulcan XC72	0.7428
Monarch 120	0.6225
Regal 250R	0.3567
Regal 500R	0.4372

C. Vulcan XC72 carbon black is suspended in several buffer solutions having different pH. Five milligrams of Vulcan XC72 carbon particles are homogenized in 2 ml of different buffer solutions containing 2% dextran 9,400 and incubated for 2 hours at ambient temperature. After the incubation, 5 μl of each homogenate are added to 1 ml of distilled water. One milliliter of 3% bovine serum albumin in the same buffer is added to the mixture which is then sonicated and incubated for approximately 12 hours at ambient temperature. At the end of the incubation, 5 μl of the mixture are suspended in 1 ml of distilled water and absorbency is measured at 700 nm. The results are as follows:

Buffers	Ionic Strength	pH	OD at 700 nm	
			Dextran	Bovine Serum Albumin
sodium phosphate	0.1 M	6.0	0.3335	0.6030
sodium phosphate	0.1 M	6.8	0.5142	0.7462
Tris-HCl	0.02 M	6.8	0.6277	0.8452
sodium phosphate	0.003 M	7.0	0.4722	0.5389
sodium phosphate	0.1 M	7.6	0.4348	0.6100
Tris-HCl	0.02 M	8.0	0.6479	0.5220
glycine-HCl	0.1 M	8.3	0.4666	0.5197
Tris-citrate	0.1 M	8.6	0.4284	0.4933

As can be seen, buffer solutions having pH values of about 6.8 to 8 are particularly good for the dispersion of carbon particles.

Example 12

To a mixture of 1 mg of anti-human chorionic gonadotropin antibody in 1 ml of 0.3 M borate buffer (pH 9.0) are added with stirring 50 µg of flourescein isothiocyanate. Stirring is continued for one hour and the mixture is then passed over a Sephadex (RTM) G-25 column to remove unreacted isothiocyanate and other unwanted materials. The ratio of antibody:isothiocyanate was approximately 1:3. To an aqueous suspension of 1 mg of carbon black (Vulcan 72) is added 0.5 mg of the antibody conjugate. The mixture is sonicated, incubated for about 12 hours at ambient temperatures, and subjected to centrifugation three times. The final pellet, suspended in a buffer such as described in Example 6, can be stored at 4°C until use.

Similar products can be obtained utilizing anti-luteinizing hormone, goat anti-human Immunoglobin G, and Immunoglobin M antibodies.

Example 13

To 10 ml of an aqueous suspension of 5 mg of carbon black (Vulcan* 72) is added 200 µl of goat anti-mouse antiserum. The mixture is sonicated and incubated for about 12 hours at ambient temperatures. There then is added 1 mg of anti-human chorionic gonadotropin antibody and this mixture is incubated for two hours at ambient temperatures and subjected to centrifugation three times. The final pellet, suspended in a buffer such as described in Example 6, can be stored at 4°C until use.

Example 14

To a suspension of 5 mg of carbon black in 10 ml of phosphate buffer solution (PBS) are added 2 mg of avidin. After incubation for two hours at ambient temperature, 5 ml of 3% bovine serum albumin in PBS are added. After standing for two hours, 0.5 mg of biotinylated anti-human chorionic gonadotropin in 1% bovine serum albumin in PBS is added. After an additional one hour incubation, the mixture is subjected to centrifugation three times. The final pellet, suspended in a buffer such as described in Example 6 and then briefly sonicated, can be stored at 4°C until use.

Example 15

Ten milligrams of Vulcan XC72 carbon particles are homogenized in 2 ml of 20 mM Tris-hydrochloride buffer (pH 6.8) containing 40 mM sodium chloride and 2% dextran 9,400. After 2 hours incubation at ambient temperature, a solution of 5 mg of flourescein isothiocyanate in 1 ml of Tris-hydrochloride buffer is added to the solution. The mixture is briefly sonicated and incubated for approximately 12 hours at ambient temperature. After incubation, 20 ml of 0.1 M sodium phosphate buffer (pH 7.6) in 0.1 M sodium

chloride are added to the carbon solution which then is centrifuged at 4°C at 15,000 RPM. This step is repeated three times and the resultant pellet suspended in 20 ml of phosphate buffer.

Two milligrams of bovine serum albumin are added to 2 ml of the above suspension and the mixture incubated for six hours and then subjected to centrifugation three times. Excess glutaraldehyde (1%) is added and after incubation for three hours at ambient temperature removed by centrifugation. A solution of 10 μg of thyroxine in sufficient dimethylformamide is added and this mixture is incubated for three hours at ambient temperature and then subjected to centrifugation three times. The final pellet, suspended in a buffer such as described in Example 6 and then briefly sonicated, can be stored at 4°C until use.

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CLAIMS

1. An immunochemical label comprising particulate carbon black on which is adsorptively immobilized a component which terminates distally from the point of adsorption with an immunologically active ligand or ligand binding molecule, for reaction between said immunological ligand or said ligand binding molecule and an analyte.

2. An immunochemical label according to claim 1 in which said component comprises the immunological ligand or ligand binding molecules covalently linked through a linking reagent and said linking reagent is adsorbed on the surface of said carbon black.

3. An immunochemical label according to claim 2, wherein the linking agent is an imide, azide, isothiocyanate, imidoester, or dialdehyde.

4. An immunochemical label according to claim 3 wherein the linking reagent is maleimide, succinimide, phenylazide, glutaraldehyde, or N-hydroxysuccinimide ester.

5. An immunochemical label according to claim 3 wherein the linking reagent is an isothiocyanate.

6. An immunochemical label according to claim 5 wherein the isothiocyanate is phenylisothiocyanate, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid, 4-N,N-dimethylaminoazobenzene-4′-isothiocyanate, fluorescein isothiocyanate, or rhodamineisothiocyanate.

7. An immunochemical label according to claim 6 wherein the linking reagent is phenylisothiocyanate.

8. An immunochemical label according to claim 6 wherein the linking reagent is 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid.

9. An immunochemical label according to claim 6 wherein the linking reagent is 4-N,N-dimethylaminoazobenzene-4′-isothiocyanate.

10. An immunochemical label according to claim 6 wherein the isothiocyanate is fluorescein isothiocyanate.

11. An immunochemical label according to claim 6

wherein the linking reagent is rhodamineisothiocyanate.

12. An immunochemical label according to any one of the preceding claims, wherein the particulate carbon black and immobilized component adsorptively immobilized thereon are coated with polyethylene glycol having a molecular weight of from 200 to 20,000 or dextran having a molecular weight of from 10,000 to 500,000.

13. An immunochemical label according to claim 12 wherein the dextran has a molecular weight of from 10,000 to 50,000.

14. An immunochemical label according to claim 12 wherein the polyethylene glycol has a molecular weight of from 5,000 to 12,000.

15. An immunochemical label according to any one of the preceding claims, wherein said component comprises the immunochemical ligand or ligand binding molecules bound to a protein and said protein is adsorbed on the surface of said carbon black.

16. An immunochemical label according to claim 15 wherein the immunological ligand or ligand binding molecules are covalently linked to said protein through an immunological bond.

17. An immunochemical label according to claim 15 wherein the immunological ligand or ligand binding molecules are covalently linked to said protein through a linking reagent.

18. An immunochemical label according to any one of claims 1 to 14, wherein said component comprises the immunological ligand or ligand binding molecules bound to a protein, said protein is covalently linked to a linking reagent and said linking reagent is adsorbed on the surface of said carbon black.

19. An immunochemical label according to claim 18 wherein the immunological ligand or ligand binding molecules are covalently linked to said protein through a second linking reagent.

20. An aqueous suspension of an immunochemical label according to any one of the preceding claims.

21. An aqueous suspension according to claim 20 including at least one buffer providing a pH at which the immobilized immunological ligand is stable and within the range of from 6 to 9.

22. An aqueous suspension according to claim 21 including at least one buffer providing a pH of from 6.5 to 8.5.

23. The method of preparing an immunochemical label according to any one of claims 3 to 19, which comprises linking immunological ligand or ligand binding molecules to the particulate carbon black by, simultaneously or sequentially, allowing a linking reagent to both (i) react covalently with the immunological ligand or ligand binding molecules and (ii) be adsorbed on finely particulate carbon black.

24. The method according to claim 23 wherein the immunochemical label is contacted with an aqueous solution of a polyethylene glycol having a molecular weight of from 100 to 20,000.

25. The method according to claim 23 wherein the finely particulate carbon black is contacted with an aqueous solution of a dextran having a molecular weight of from 10,000 to 50,000.

26. The method according to claim 23, 24 or 25, wherein the immunological ligand or ligand binding molecules are linked to the particulate carbon black through an imide, azide, isothiocyanate, imidoester, or dialdehyde.

27. The method according to claim 26, wherein the immunological ligand or ligand binding molecules are linked to the particulate carbon black through phenylisothiocyanate, 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid, 4-N,N-dimethylaminoazobenzene-4'-isothiocyanate, fluorescein isothiocyanate, or rhodamineisothiocyanate.